In re Appln. of Brandt et al.
Application No. Unassigned (National Phase of PCT/EP03/04037)

CLAIM AMENDMENTS

1. (Currently Amended) Method for the detection and characterisation of primary tumours and separate areas of primary tumours, respectively, ~~. The characteristic feature of this~~ method comprising using ~~is that~~ sample material ~~is used~~ to isolate and concentrate cell clusters of tumour cells, followed by an analysis of the genetic changes in these isolated cell clusters.

2. (Currently Amended) Method according to ~~the above~~ claim 1, ~~Characterisation: The~~ wherein the sample material consists of cell cultures, blood, urine, nipple aspiration fluid from the female breast or tissue from primary tumours.

3. (Currently Amended) Method according to ~~one of the preceding claims~~ claim 1, ~~Characterisation:~~ wherein ~~Polymorphic~~ polymorphic DNA of primary tumours or separate areas of primary tumours, and alterations therein, respectively, are recorded and compared with corresponding polymorphic DNA of cell clusters, and alterations therein, respectively.

4. (Currently Amended) Method according to ~~one of the preceding claims~~ claim 1, ~~Characterisation: The~~ wherein DNA of the following polymorphic sequences are analysed: D7S522, D8S133, D8S258, D8S265, NEFL, D10S541, D10S1765, D10S579, D13S153, D16S400, D16S402, D16S413, D16S422, p53, BB1, BB2, CAII, CAIII, CAIV, CAV and/or D17S855.

5. (Currently Amended) Method according to ~~one of the preceding claims~~ claim 1, ~~Characterisation: The~~ wherein the polymorphic DNA is reproduced before analysis.

6. (Currently Amended) Method according to ~~the preceding~~ claim 5, ~~Characterisation: The~~ wherein the polymorphic DNA of three polymorphic sequences, D7S522, D8S256, D16S400 or NEFL, D13S153, D17S855 or D10S541, D16S402, D16S422 are analysed together and/or reproduced.

7. (Currently Amended) Method according to ~~the preceding~~ claim 6, ~~Characterisation: The~~ wherein the polymorphic DNA is reproduced prior to analysis by polymerase chain reaction (PCR).

8. (Currently Amended) Method according to ~~the preceding~~ claim 7, ~~Characterisation: The~~ wherein the polymorphic DNA is reproduced by using the following primer pairs:

GCAGGACATGAGATGACTGA and GTTATGCCACTCCCTCACAC (for D7S522);
GTTTGAAGAATTTGAGCCAACC and TTCTTCTGCACACTTGGCAC (for BB1+2);
CTCGAGGTCTCATCCTCTTTCC and GCAGAGGTGCACAAAGGAGTAA (for CAII);
AGGCCCACAGAGGAGATAACAG and CAGGTGTGGTAGATGCCAAAGA (for CAIII);
GCAACTTATCCAAACCCTGACC and AGAGTGGACTAGGAAATGCTAGGAG (for CAIV);
AGTTCCTGACTGGGAATTCGAT and TTGGCCAAATTACACACCTTTG (for CAV);
TTCCATTTGTCTCGGTT and AGTCTCCTCGTCTCACACCT (for D7S2550);
CAGTGCTGGAGTTGTTCAAG and CTGGGAGTCAAGTGTTTTGG (for D7S2429);
TGCTAAGTCTTGATTTTGCC and AACGGTCATCTGTGTTCG (for D7S2467);
GGTGTTTGTGTCATTACGCT and TTTGCTGTAGAGGATGCAAT (for D7S478);
TTCGGGCTCTCTGTTATAAA and CCGAAGCAGGATTTTATTTC (for D7S670);
AGCTGCCAGGAATCAACTGAGAG and GATGCTCACATAAAGGAGGGAGG (for D8S258);
CCAATACCTGCAGTAGTGCC and GAGCTGCTTAACACATAGGG (for NEFL);
CACCACAGACATCTCACAACC and CCAGTGAATAGTTCAGGGATGG (for D10S541);
AGGGTTATGTATAACCGACTCC and GTCTAAGCCCTCGAGTTGTGG (for D13S153);
GGTTCACAATTGGACAGTAT and GAACCCTCCATGCTGACATT (for D16S400);
GTACCCATGTACCCCCAATA and CAAAGCACCACATAGACTAA (for D16S402);
GAGAGGAAGGTGGAAATACA and GTTTAGCAGAATGAGAATAT (for D16S422);
AATAAATTCCCACTGCCACTC and ATCCCCTGAGGGATACTATTC (for p53);
GGATGGCCTTTTAGAAAGTGG and ACACAGACTTGTCCTACTGCC (for D17S855).

9. (Currently Amended) Method according to ~~one of the claims~~ claim 5, ~~8. Characterisation: The~~ wherein the reproduced DNA fragments are split and analysed by capillary electrophoresis.

10. (Currently Amended) Method according to ~~one of the above claims~~ claim 1, ~~Characterisation: For~~ wherein the isolation or concentration of tumour cells cytokeratin-positive cells were isolated from sample material, and/or positive epithelial cells for tissue specific proteins.

11. (Currently Amended) Method according to ~~the preceding~~ claim 10, ~~Characterisation: Epithelial~~ wherein epithelial cells are concentrated from sample material by means of density gradient centrifugation-if necessary after homogenisation in a solvent, ~~Cytokeratin-~~ and cytokeratin-positive and/or positive cell clusters from tissue specific proteins are then split off by means of immunomagnetic cell isolation.

12. (Currently Amended) Method according to ~~the preceding~~ claim 11, ~~Characterisation: The~~ wherein the medium for the density gradient centrifugation is a hyper-osmotic medium.

13. (Currently Amended) Method according to ~~the preceding~~ claim 12, ~~Characterisation: The~~ wherein the hyper-osmotic buffer consists of one of the following mediums: 13.8% (w/v) Diatrizoate and 8% (w/v) dextran 500 in H_2O (polymorphprep) or 13% (w/v) *Nycodenz*, 0.58% (w/v) NaCl and 5 mM Tricine-NaOH pH 7.4 in H_2O (Nycoprep).

14. (Currently Amended) Method according to ~~one of the preceding claims~~ claim 1, ~~Characterisation: Genetic~~ wherein genetic changes in the isolated cell clusters are analysed by means of cluster analysis.

15. (Currently Amended) Application of a method according to ~~one of the preceding claims~~ claim 1 for the molecular characterization of tumours or tumour sections or for the determination of clonality from cells clusters isolated from sample material as well as for the detection of a tumour to determine the tumour stage, the metastasising potential, therapy requirements, efficacy of therapy of a tumour or part thereof, as well as the assessment of the course of a disease or therapy.

16. (Currently Amended) Application according to ~~the preceding~~ claim 15 for the detection and/or characterisation of tumours or tumour areas of the following carcinomas: mamma-, ovarial-, colon-, gastric-, prostate and/or bladder carcinoma.